

Identification of New, Odor-Active Thiocarbamates in Cress Extracts and Structure–Activity Studies on Synthesized Homologues

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New, odorant nitrogen- and sulfur-containing compounds are identified in cress extracts. Cress belongs to the botanical order Brassicales and produces glucosinolates, which are important precursors of nitrogen- and sulfur-containing compounds. Those compounds often present low perception thresholds and various olfactive notes and are thus of interest to the flavor and fragrance chemistry. When the study of organonitrogen and organosulfur compounds is undertaken, Brassicale extracts are one of the matrices of choice. Cress extracts were studied by analytical (GC-MS, GC-FPD) and chemical (fractionation) means to identify new interesting odorant compounds. Two compounds that have never been reported in cress extracts, containing both nitrogen and sulfur, were discovered: *N*-benzyl *O*-ethyl thiocarbamate and *N*-phenethyl *O*-ethyl thiocarbamate. These two molecules being of organoleptic interest, their homologues were synthesized and submitted to organoleptic tests (static and GC–sniffing). Their odors evolve from garlic and onion over green, mushroom- and cress-like to fresh, spearmint-like. This paper presents the origin, chemical synthesis, and organoleptic properties of a series of *O*-alkyl thiocarbamates.

KEYWORDS: Thiocarbamates; organonitrogen/organosulfur compounds; *Nasturtium*; GC–sniffing; odor evaluation; flavor; absolute; GC-MS; Brassicales

INTRODUCTION

Flavor and fragrance research seeks new flavoring molecules to satisfy consumers' demands and to create new odors and flavors. Extracts of natural matrices such as essential oils, concretes, and absolutes are an inexhaustible source of odorant molecules. Flavor research thus turns to natural matrices for the identification of new flavoring substances. Due to their frequent low perception thresholds, organonitrogen and organosulfur compounds are of particular interest. Their presence in a matrix, even in very low quantities, can considerably influence the matrix's organoleptic properties. A great number of such compounds have already been discovered in the past, but further studies of natural extracts benefit from the constant improvement in analytical techniques and lead to the identification of an increasing number of compounds (1–3).

In terms of flavor and fragrance research, it is often useful to combine physical [universal, MS, FID; and specific, flame photometric detector (FPD), pulsed flame photometric detector (PFPD), atomic emission detector (AED), nitrogen–phosphorus detector (NPD), sulfur chemiluminescence detector (SCD)]

detectors with physiological/sensorial detectors such as the human nose. These olfactometric techniques, especially gas chromatography–olfactometry (GC-O), render GC separation and evaluation of interesting odorant compounds possible, whereas MS allows molecular identification. Specific detectors can, in certain cases, contribute to the detection of compounds not detected by universal detectors.

The matrices chosen for this study are two edible cress species belonging to the botanical order Brassicales (4): *Tropaeolum majus* L. (Indian cress or *Nasturtium*), which belongs to the Tropaeolaceae family, and *Nasturtium officinale* R.BR. (watercress), which belongs to the Brassicaceae family. Brassicale plants are known for the production of glucosinolates, the precursors of isothiocyanates, cyanides, and thiocyanates (Figure S 1) (5).

Watercress, the botanical name of which, *Nasturtium officinale* R.BR., is an allusion to its spicy flavor and comes from the Latin words *nasum* (nose) and *torquere* (to grimace) (6), is about 30–80 cm tall and grows naturally next to rivers, fountains, and humid areas. It is known for its high content in vitamins A and C (7), and its main glucosinolate is gluconasturtiin (phenethyl glucosinolate) (8).

Indian cress is also called *Nasturtium*, as an allusion to its spicy flavor. The plant grows preferably in sunny places and

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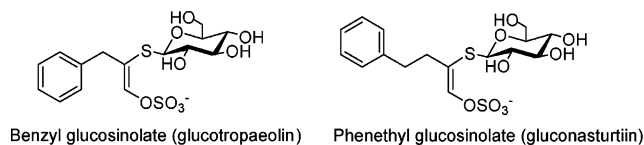


Figure 1. Benzyl glucosinolate (glucotropaeolin) and phenethyl glucosinolate (gluconasturtiin) (5).

up to an approximative height of 40 cm. Its main glucosinolate is glucotropaeolin (benzyl glucosinolate) (5). The two main glucosinolates of the two cress species are represented in **Figure 1**.

The aim of this work is the identification of molecules with interesting organoleptic properties in cress extracts with focus on organonitrogen and organosulfur compounds.

MATERIALS AND METHODS

Chemicals and Extracts. *Botanical Source and Plant Material.* Commercial Indian cress absolute was provided by Robertet S.A., Grasse, France. It had been produced in Rustenburg, South Africa, by extraction of the fresh plant with *n*-hexane followed by treatment with ethanol.

Watercress was purchased from Cresson de Provence, Hyères, France, in April 2006. A voucher specimen was deposited at the Herbarium of the Botanical Garden of Nice (voucher B-9298). Commercial watercress (Florette, Lessay, L'Isle-sur-Sorgue, France) stored under plastic packaging was purchased at a supermarket in Nice, France, in June 2005.

NMR. NMR was carried out on a Bruker Avance AC200 (200 MHz) and DRX500 (500 MHz) (Bruker, Wissembourg, France). The solvent was CDCl₃ (Eurisotop, D007H); chemical shifts (δ) are given in parts per million (ppm), and coupling constants (*J*) are given in hertz. Thiocarbamates present two rotamers forms in NMR.

Elementary Analysis. Compounds with no known CAS Registry Number (CAS RN) were submitted to elementary analysis at the Service Central d'Analyse du CNRS, Service Central d'Analyse, Vernaison, France.

Melting Point. The melting point of solid compounds was determined on an Electrothermal 9100 apparatus and is given in degrees centigrade.

Chemicals. All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France), Merck (Fontenay-sous-Bois, France), VWR Prolabo (Fontenay-sous-Bois, France), or Eurisotop (Saint Aubin, France): allyl isothiocyanate (Aldrich, 377430), benzyl isothiocyanate (Aldrich, W510548), ethyl isothiocyanate (Aldrich, E33904), 3-(methylthio)propyl isothiocyanate (Aldrich, W331201), phenethyl isothiocyanate (Aldrich, W401404), absolute ethanol (Merck, 100983), 1-propanol (Fluka, 82092), methanol (Riedel-de Haën, 34860), diethyl ether (Riedel-de Haën, 24004), methylene chloride (Riedel-de Haën, 24233), pentane (Riedel-de Haën, 32288), petroleum ether (Riedel-de Haën, 24587), hexane (Riedel-de Haën, 15671), cyclohexane (Riedel-de Haën, 15329), and ethanol 96% (APC, 200-578-6). Solvents were distilled prior to use.

Plant Extraction. Fresh watercress (2300 g; leaves and stems, cut into pieces; origin, Hyères) were extracted with 3.6 L of *n*-hexane during 6 h (agitation, *T* = 62–63 °C) to give, after filtration, treatment with MgSO₄ (VWR, 25162.361), and solvent evaporation, 2.48 g (yield = 0.11%) of concrete. Extraction was carried out on the same day as the harvest.

Watercress (89.60 g; leaves and stems, cut into pieces, from a supermarket) were extracted in a Soxhlet apparatus with 400 mL of cyclohexane during 4 h to give, after filtration, treatment with MgSO₄, and solvent evaporation, 0.05 g (yield = 0.06%) of concrete.

Fractionation. Fractionation was performed according to the literature (9, 10). Indian cress absolute (20.03 g) was solubilized in 100 mL of diethyl ether. The ethereal solution was washed three times with 50 mL of HCl 1 N (Riedel-de Haën, 30721). The aqueous phases were united (F₁), extracted with ether (F₂), and treated with NaOH (Sigma-Aldrich, 221465) 1 N solution until pH 9. After ethereal extraction and drying over MgSO₄, 0.09 g of a (basic) fraction F_{1,1} was obtained.

The ethereal fraction (F₂) was washed three times with 50 mL of saturated K₂CO₃ (Aldrich, 209619) solution and gave, after drying over MgSO₄, 19.90 g of neutral fraction F_{2,3}. The aqueous phase (pH 10) was acidified with HCl 1 N until pH 2 and extracted with ether and gave, after drying over MgSO₄, 0.04 g of (acid) fraction F_{2,4}.

Silica gel column chromatography (open column, 150 g of silica gel 60, 40–63 μ m; Merck, 109385) of 4.51 g of fraction F_{2,3} was realized to isolate the unknown major compound (gradient from 100% *n*-pentane to 100% diethyl ether). The unknown compound was isolated after further silica gel column fractionation of the elution fraction 50% *n*-pentane/50% diethyl ether (gradient from 100% pentane to 100% diethyl ether) by using 80% *n*-pentane/20% diethyl ether. All obtained fractions were analyzed by GC-MS and GC-FID (**Scheme 1**).

Analytical Studies. **GC-MS.** GC-MS analysis was carried out using a HP 5890 series II GC coupled to a HP 5971A MS (Hewlett-Packard, Massy, France). Samples were analyzed on a fused-silica capillary column HP-1 (polydimethylsiloxane, 50 m \times 0.20 mm i.d. \times film thickness = 0.50 μ m; Interchim, Montluçon, France) and HP-20M (polyethyleneglycol, 50 m \times 0.20 mm i.d. \times film thickness = 0.33 μ m; Interchim): carrier gas, helium; constant pressure, 220 kPa; injector temperature, 230 °C; split ratio, 1:100; temperature program, raised from 60 to 250 °C at 2 °C/min and then held isothermal (120 min) at 250 °C (apolar column) or 220 °C (polar column); ion source temperature, 230 °C; transfer line temperature, 280 °C (apolar column) or 230 °C (polar column); ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range of 35–400 amu.

Component Identification. Identification of the constituents was based on computer matching against commercial libraries (Wiley, MassFinder 2.1 Library, NIST98), laboratory mass spectra libraries built from pure substances, and MS literature data (11–14) combined with comparison of GC retention indices (RI) on apolar and polar columns. RIs were calculated with the help of a series of linear alkanes C6–C26 and C21–C40 on apolar and polar columns (HP-1 and HP-Innowax). Compounds available in the laboratory were confirmed by external standard compound co-injection.

Quantification. Quantification was performed on an Agilent HP 6890N GC (Agilent, Massy, France) equipped with a fused-silica capillary column HP-1 (polydimethylsiloxane, 50 m \times 0.20 mm i.d. \times film thickness = 0.33 μ m; Interchim) and a FID: carrier gas, helium; constant flow, 1 mL/min; injector and detector temperatures, 250 °C; split ratio, 1/10; temperature program, raised from 60 to 230 °C at 2 °C/min and then held isothermal (120 min).

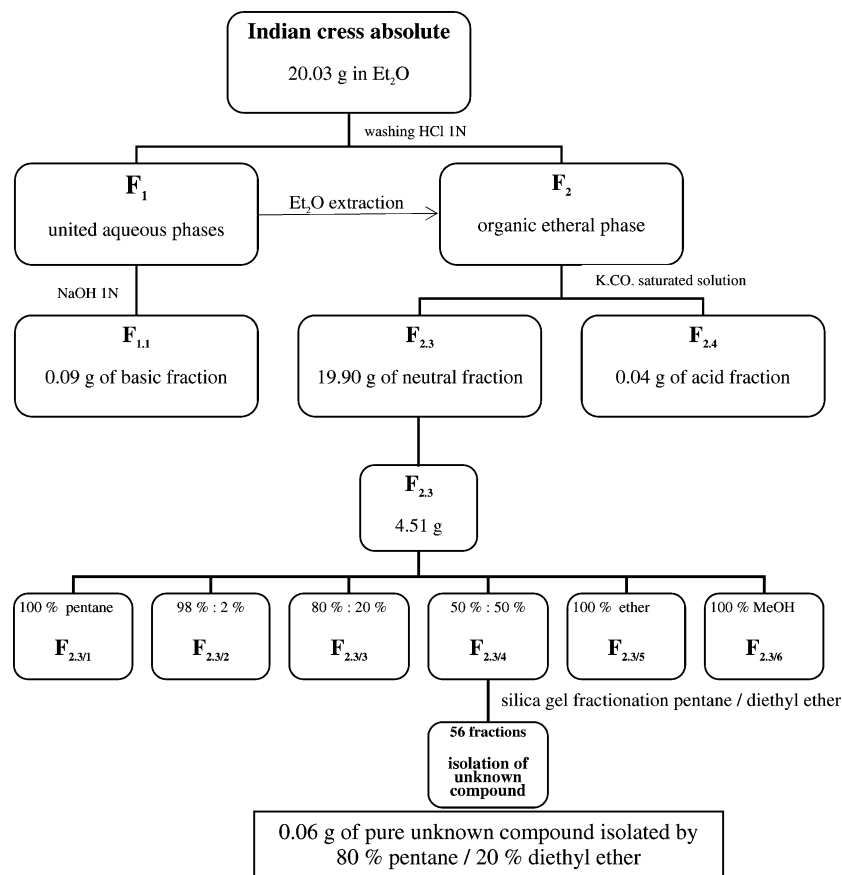
An external standardization method was used. Linearity of the calibration curve was found for concentrations between 0.124 and 0.962 mg/g. For each compound, five solutions of different equidistant concentrations were prepared. For concentrations close to the lower and to the upper limit, 10 GC-FID injections were realized, 2 for the others. Homogeneity of variances was verified by statistic bilateral Fisher test by taking homogeneity of variances as *H*₀ (15).

Linearity of the calibration function was tested by comparison of the calibration function of first order to the calibration function of second order to prove that the diminution of the residual variance was significant. The nonsignificance of residual variance was taken as *H*₀ (16).

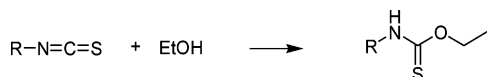
Specific Detection. Detection of organosulfur analytes was carried out on a HP 5990 series II GC equipped with a FID and a FPD. Fused-silica capillary column: HP-1 (polydimethylsiloxane, 50 m \times 0.20 mm i.d. \times film thickness = 0.33 μ m; Agilent, Massy, France); carrier gas, nitrogen; constant pressure, 220 kPa; detector gas (flow), nitrogen (1.08 mL/min), hydrogen (75 mL/min), air (100 mL/min); injector temperature, 230 °C; detector temperature, 250 °C; splitless mode; temperature program, raised from 60 to 250 °C at 2 °C/min and then held isothermal (120 min).

GC-Olfactometry. GC-O analysis was performed on a Shimadzu GC-2010 GC (Shimadzu, Champs-sur-Marne, France) equipped with an automatic injector type AOC-20i, a FID and an ATAS olfactory port OP275 with a glass nasal cone (ATAS, Veldhoven, The Netherlands): column, Equity-5 (30 m \times 0.25 mm i.d. \times film thickness = 0.25 μ m; Supelco, L'Isle d'Abeau, France); carrier gas, nitrogen; constant flow, 0.8 mL/min; injector temperature, 250 °C; detector temperature, 250 °C; split ratio, 1:100; transfer line for nearly

Scheme 1. Fractionation of Indian Cress Absolute



Scheme 2. Formation of Thiocarbamates in Brassicales and during Absolute Preparation



simultaneous detection, length transfer line, inside oven, 0.89 m; outside oven, 1.49 m; temperature transfer line, 250 °C. Sixty percent of the flow was directed to the FID while 40% was directed into the heated sniffing port. The temperature program was 200 °C isothermal. Samples were solubilized at a concentration of 5% in diethyl ether. Two trained panelists were selected for odor evaluation (17).

Static Evaluation. Static evaluation was carried out at Robertet S.A., Grasse, France, by two flavor chemists. Samples were prepared at a concentration of 1% in EtOH 96% followed by dilution to 10 ppm (15 ppm for two samples) in water.

Synthesis. Synthesis of Thiocarbamates. Ten millimoles of isothiocyanate and 50 mmol of anhydrous alcohol (methanol, ethanol, or 1-propanol) were heated to reflux for 50 h under nitrogen atmosphere. The mixture was dried over MgSO_4 and the alcohol evaporated. The crude product was purified by silica gel column chromatography (open column, silica gel 60, 40–63 μm ; Merck, 109385) using 100% petroleum ether followed by 80% petroleum ether/20% diethyl ether as solvents and the corresponding thiocarbamate gave yields between 25 and 96% with a GC purity range of 97–99%.

Five different isothiocyanates known for their presence in Brassicales plants were used: allyl isothiocyanate, benzyl isothiocyanate, ethyl isothiocyanate, 3-(methylthio)propyl isothiocyanate, and phenethyl

isothiocyanate (5). Except for ethyl isothiocyanate, all isothiocyanates are listed as positive flavoring substances in the *Official Journal of the European Communities* published in January 2002 (18). The use of educts listed as positive does not automatically lead to an approval of the product as a flavoring compound, but it is a criterion of choice when flavoring molecules are synthesized in order to choose between the numerous educts possible.

Synthesized Thiocarbamates. *N*-Allyl *O*-methyl thiocarbamate: $\text{C}_5\text{H}_9\text{NOS}$ (MW 131 g/mol); CAS RN 41596-55-8 (19); liquid compound; yield 58%.

N-Benzyl *O*-methyl thiocarbamate: $\text{C}_9\text{H}_{11}\text{NOS}$ (MW 181 g/mol); CAS RN 65263-72-1 (20); solid compound; mp 44–46 °C; yield 25%.

N-Ethyl *O*-methyl thiocarbamate: $\text{C}_4\text{H}_9\text{NOS}$ (MW 119 g/mol); CAS RN 65351-53-3 (21); liquid compound; yield 57%.

N-3-(Methylthio)propyl *O*-methyl thiocarbamate: $\text{C}_6\text{H}_{13}\text{NOS}_2$ (MW 179 g/mol); new compound; liquid compound; yield 31%.

N-Phenethyl *O*-methyl thiocarbamate: $\text{C}_{10}\text{H}_{13}\text{NOS}$ (MW 195 g/mol); new compound; liquid compound; yield not determined.

N-Allyl *O*-ethyl thiocarbamate: $\text{C}_6\text{H}_{11}\text{NOS}$ (MW 145 g/mol); CAS RN 817-97-0 (22, 23); liquid compound; yield 80%.

N-Benzyl *O*-ethyl thiocarbamate: $\text{C}_{10}\text{H}_{13}\text{NOS}$ (MW 195 g/mol); CAS RN 55365-86-1 (20, 24); liquid compound; yield 79%.

N-Ethyl *O*-ethyl thiocarbamate: $\text{C}_5\text{H}_{11}\text{NOS}$ (MW 133 g/mol); CAS RN 998-98-1 (25); liquid compound; yield 74%.

N-3-(Methylthio)propyl *O*-ethyl thiocarbamate: $\text{C}_7\text{H}_{15}\text{NOS}_2$ (MW 193 g/mol); CAS RN 89855-25-4 (26); liquid compound; yield 82%.

N-Phenethyl *O*-ethyl thiocarbamate: $\text{C}_{11}\text{H}_{15}\text{NOS}$ (MW 209 g/mol); CAS RN 55365-88-3 (27); liquid compound; yield 62%.

N-Allyl *O*-propyl thiocarbamate: $\text{C}_7\text{H}_{13}\text{NOS}$ (MW 159 g/mol); CAS RN 89895-26-1 (28, 29); liquid compound; yield 81%.

N-Benzyl *O*-propyl thiocarbamate: $\text{C}_{11}\text{H}_{15}\text{NOS}$ (MW 209 g/mol); new compound; liquid compound; yield 96%.

N-Ethyl *O*-propyl thiocarbamate: $\text{C}_6\text{H}_{13}\text{NOS}$ (MW 147 g/mol); new compound; liquid compound; yield 93%.

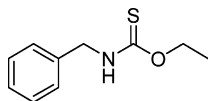
Figure 2. Molecular structure of *N*-benzyl *O*-ethyl thiocarbamate identified in Indian cress absolute.

Table 1. *O*-Alkyl Thiocarbamates Synthesized and Their Retention Indices, MS Data, and Organoleptic Properties

| <div> <div>compound</div> $\begin{array}{c} \text{S} \\ \parallel \\ \text{R}_1\text{-N}-\text{C}-\text{O}-\text{R}_2 \\ \\ \text{H} \end{array}$ </div> | odor evaluation | | | |
|---|-----------------|---|---|-----------------------------------|
| | RI ^a | EI-MS fragmentation <i>m/z</i> (%) | static ^b (10 ppm in water) | dynamic (GC-O) |
| R ₂ = methyl R ₁ = allyl | 1115/1948 | 116(100), 131(55) [M ⁺], 56(26), 75(17), 41(11), 39(11), 58(8), 88(8), 60(8), 98(7) | garlic, Camembert cheese, cabbage | garlic |
| R ₁ = benzyl | 1608/2766 | 181(100) [M ⁺], 106(89), 91(76), 79(48), 166(45), 65(22), 123(20), 77(19), 182(11), 51(10) | green, sulfury, earthy, mushroomy, faint odor | green, faint odor |
| R ₁ = ethyl | 1046/1312 | 119(100) [M ⁺], 44(47), 104(32), 60(23), 58(15), 75(13), 88(8), 61(7), 59(7), 120(6) | sulfury, onion/garlic, cabbage | rubber, plastic, grilled |
| R ₁ = 3-(methylthio)propyl | 1556/2667 | 132(100), 179(62) [M ⁺], 75(49), 61(26), 58(20), 41(14), 73(13), 146(13), 72(12), 47(10) | green, mushroom, earthy, cress | green, cress |
| R ₁ = phenethyl | 1703/2827 | 104(100), 195(43) [M ⁺], 91(17), 105(15), 75(13), 103(11), 77(9), 65(6), 78(6), 44(5) | fresh, mushroom, sulfury, rubber | mushroom |
| R ₂ = ethyl R ₁ = allyl | 1195/1979 | 145(96) [M ⁺], 130(68), 116(75), 102(27), 100(18), 88(19), 60(25), 56(100), 41(54), 39(35) | garlic, sulfury, pungent, slightly plastic note, mains gaz, strong odor | garlic, cooked garlic, onion |
| R ₁ = benzyl | 1687/2784 | 195(38) [M ⁺], 166(29), 123(12), 106(61), 92(10), 91(100), 60(24), 79(31), 77(19), 65(22), 51(11) | green, cress, bitter, faint odor, fruity, honey ^c | green, cress, mild, faint odor |
| R ₁ = ethyl | 1121/1878 | 44(100), 133(94) [M ⁺], 72(45), 60(41), 104(21), 88(17), 61(11), 59(9), 42(8), 56(8) | grilled, rubber, plastic, earthy, cabbage | grilled, a little bitter |
| R ₁ = 3-(methylthio)propyl | 1620/2696 | 193(83) [M ⁺], 146(100), 118(27), 89(13), 73(11), 61(42), 58(25), 56(14), 45(12), 41(16) | green, cress, fresh mushroom, metallic | green, cress, radish, pungent |
| R ₁ = phenethyl | 1773/2847 | 209(37) [M ⁺], 120(13), 105(23), 104(100), 103(15), 91(30), 79(7), 78(8), 77(15), 65(12) | astringent, cress, green, black radish, spicy, curry, leek | mushroom, green, moss, wet forest |
| R ₂ = propyl R ₁ = allyl | 1286/2069 | 41(100), 56(77), 159(56) [M ⁺], 102(49), 117(44), 39(43), 84(27), 43(22), 116(17), 60(14) | garlic/sulfur, rotten onion, burned plastic, strong | spearmint |
| R ₁ = benzyl | 1784/2868 | 91(100), 106(30), 209(26) [M ⁺], 166(25), 79(15), 167(13), 65(11), 77(9), 92(9), 41(7) | sulfury, fruity, honey-like ^c | green, floral, almond-like |
| R ₁ = ethyl | 1219/1970 | 72(100), 44(98), 147(96) [M ⁺], 60(44), 106(39), 41(31), 88(25), 43(22), 105(16), 39(15) | garlic, sulfury, onion, leek, green | green, plastic |
| R ₁ = 3-(methylthio)propyl | 1730/2770 | 207(100) [M ⁺], 118(92), 61(62), 160(53), 41(50), 88(42), 89(42), 58(35), 56(27), 132(23) | cress, earthy, sulfury, geranium, green, slightly garlic/onion, very strong | green |
| R ₁ = phenethyl | 1875/2929 | 104(100), 105(36), 91(25), 223(22) [M ⁺], 181(16), 103(11), 77(10), 90(8), 120(8), 65(7) | green-spicy, earthy, rocket, winter-cress, leek | mushroom |

^a Retention indices as determined on HP-1 and HP-Innowax using the homologous series of *n*-alkanes. ^b Sample preparation: 1% in EtOH 96% followed by dilution to 10 ppm in water. ^c Evaluated at 15 ppm in water.

N-3-(Methylthio)propyl *O*-propyl thiocarbamate: C₈H₁₇NOS₂ (MW 207 g/mol); new compound; liquid compound; yield 79%.

N-Phenethyl *O*-propyl thiocarbamate: C₁₂H₁₇NOS (MW 223 g/mol); new compound; liquid compound; yield 60%.

RESULTS AND DISCUSSION

Cress solvent extracts are complex matrices dominated by the presence of one or two major compounds.

The major compounds contained in Indian cress absolute were benzaldehyde (0.3% of the total GC area), *N*-(phenylmethylene)-

N-benzylamine (0.7%; structure represented in Figure S 2), ethyl linolenate (0.9%), ethyl palmitate (1.0%), benzyl cyanide (1.1%), *N*-benzyl *O*-ethyl carbamate (3.8%), benzyl isothiocyanate (3.9%), and a compound that constituted 83.7% of the total GC area (chromatogram presented in Figure S 3). This previously unknown compound could not be identified using MS and retention indices on polar and apolar columns alone. To isolate and identify this analyte, and to be able to identify minor compounds, we proceeded to a series of fractionation steps. The isolation of the unknown compound allowed its structural

analysis. MS data of the analyte (Figure S 4) show a molecular mass of 195 amu, indicating the presence of nitrogen and the presence of sulfur by isotopic peaks $M + 1$ and $M + 2$. GC-FPD confirmed the presence of sulfur atom(s). This information, combined with ^1H and ^{13}C NMR (Figure S 5) allowed the identification as *N*-benzyl *O*-ethyl thiocarbamate (**Figure 2**).

Unlike essential oils, solvent extracts contain high molecular weight products, which may not be eluted in GC. Thus, one cannot conclude from the GC percentage information obtained by internal normalization the quantity of a compound actually present in an extract. To provide more precise information on the quantity of *N*-benzyl *O*-ethyl thiocarbamate in Indian cress absolute, quantification was necessary. We thus proceeded to a quantification of four selected compounds, which are either major compounds of the volatile fraction or known for their influence on organoleptic properties of Brassicales: methyl trisulfide, benzyl cyanide, benzyl isothiocyanate, and *N*-benzyl *O*-ethyl thiocarbamate (Table S 1). *N*-Benzyl *O*-ethyl thiocarbamate presents 83.7% of the total GC profile, but quantification gave more accurate information about its real quantity in the absolute: The absolute contains 35% m/m, that is, 350 mg/g, of *N*-benzyl *O*-ethyl thiocarbamate.

This compound was formerly isolated and identified by Migirab et al. in 1977 in an African medicinal Brassicale, *Pentadiplandra brazzeana* Baillon, belonging to the Pentadiplandraceae family. Migirab et al. isolated and identified four other thiocarbamates in *P. brazzeana* Baillon: *N*-benzyl *O*-methyl thiocarbamate, *N*-4-methoxybenzyl *O*-methyl thiocarbamate, *N*-4-methoxybenzyl *O*-isopropyl thiocarbamate, and *N*-phenyl *O*-(2-dimethylamino)ethyl thiocarbamate. As the authors state, the extraction solvent was CHCl_3 , and the five thiocarbamates identified were thus not artifact products formed by the extraction procedure, but naturally present in the plant material (20). To date, at least to the knowledge of the authors, there is no communication on the use of thiocarbamates in flavor and fragrance chemistry.

Because the main glucosinolate degradation product of Indian cress is benzyl isothiocyanate and the extract studied was an absolute, that is, a solvent extract treated with ethanol, we assume that the *N*-benzyl *O*-ethyl thiocarbamate was formed via additive reaction between benzyl isothiocyanate and ethanol (**Scheme 2**). To verify this hypothesis, the reaction under absolute preparation conditions (EtOH 96%, atmospheric pressure, and heating to alcohol reflux 80 °C) between benzyl isothiocyanate and ethanol was tested and resulted indeed in the formation of *N*-benzyl *O*-ethyl thiocarbamate.

Globally, the GC analysis of the different fractions of Indian cress absolute allowed the identification of a total amount of 145 compounds including 25 nitrogen-containing and 17 sulfur-containing compounds.

Watercress concrete was studied to ascertain the origin of the thiocarbamate, being either a natural compound present in the plant extract, an artifact product, or a contamination. Analysis of concrete prepared from commercially available watercress (stored under plastic packaging) led to the identification of phenethyl isothiocyanate as a major compound. The corresponding *N*-phenethyl *O*-ethyl thiocarbamate was present at about 0.4% of the total GC area in the concrete. Its quantity increased in absolute preparation conditions (treatment of the concrete with ethanol). Concrete prepared from fresh watercress also contained phenethyl isothiocyanate as a major compound. The corresponding thiocarbamate was not detected, but its formation was observed during absolute preparation.

These results confirm the observations made by Migirab et al.: whereas higher quantities of *O*-ethyl thiocarbamates are formed as major compounds during absolute preparation by additive reaction between isothiocyanates and ethanol, traces occur naturally in Brassicales, possibly formed by fermentation during storage. According to European Council Directive 88/388/CE and the *Code of Federal Regulations* of the U.S. Food and Drug Administration, solvent extracts are considered as natural and so are the thiocarbamates identified in them (30, 31).

Because *N*-benzyl *O*-ethyl thiocarbamate is an odorant compound with the typical odor of Indian cress absolute, the synthesis of its volatile homologues of both potentially natural (*O*-ethyl thiocarbamates) and synthetic origin (*O*-methyl and *O*-propyl thiocarbamates) seemed to be interesting.

O-Alkyl thiocarbamates have never been reported as odorant compounds. Migirab et al. reported the use of tuberacle extracts of *P. brazzeana* Baillon in African traditional medicine against stomach aches and confirmed the bactericidal properties of certain thiocarbamates (20). Thiocarbamates are also described in the literature as additives in insect repulsive agents (32), fungicides (33), herbicides (34), or additional compounds in antifouling agents with low toxicity to the human body (23). Little information is available on the toxicity of *O*-alkyl thiocarbamates. The thiocarbamates commonly used as pesticides or herbicides are *S*-alkyl thiocarbamates (carbonyl group), in contrast to the *O*-alkyl thiocarbamates (thiocarbonyl group) on which we report here. The toxicity of *S*-alkyl thiocarbamates to humans is reported as relatively low: LD₅₀ values exceed 1000 mg/kg except for molinate and diallate, which have LD₅₀ values of 369 and 395 mg/kg, respectively (35, 36). Possible applications as flavoring compounds must be preceded by serious toxicological studies. To evaluate the organoleptic properties, a series of *O*-alkyl thiocarbamates was synthesized. *O*-Alkyl thiocarbamates were easily obtained from the corresponding isothiocyanates and alcohol. Yields and purity are satisfactory, with yields between 25 and 96% and GC purity between 97 and 99%. Their organoleptic properties go from garlic and onion notes over mushroom, cress, and plastic odors to spearmint-like notes (**Table 1**). For some molecules, a difference between the static and the dynamic odor perception occurs. This is most probably due to the formation of hydrogen sulfide in contact with water and is a problem for static organoleptic evaluations.

O-Alkyl thiocarbamates were identified as a new class of odorant compounds via chemical and olfactive analysis of two extracts of different cress species, both being Brassicales. These compounds are formed in two main ways: Trace amounts are produced in the plant by reaction of the present isothiocyanates with ethanol from fermentation. High quantities are formed during absolute preparation, for example, treatment of the concrete with ethanol.

Organoleptic evaluation of a series of *O*-alkyl thiocarbamates was realized by static and dynamic techniques, and the odorant notes as well as the organoleptic potency of the analytes were found to be interesting. As their toxicity to humans is reported to be low, there might be a possibility for a future use of these compounds in flavor and fragrance chemistry, especially for salty, garlic/onion, mushroom, and spicy notes.

The identification of *O*-alkyl thiocarbamates in cress extracts shows that natural matrices still are an important source of new compounds. As revealed, the extraction mode can strongly

influence the chemical composition of the abstract. A detailed analytical study of the two cress extracts allowed composition elucidation of these matrices. The results comprising GC-olfactometry will be presented in the future to introduce further odorant molecules of natural origin.

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Supporting Information Available: Figure S 1, degradation of glucosinolates and resulting products; Figure S 2, structure of *N*-(phenylmethylene)-*N*-benzylamine; Figure S 3, GC-MS profile of Indian cress absolute; Figure S 4, MS spectrum of *N*-benzyl *O*-ethyl thiocarbamate; Figure S 5, NMR ¹H spectrum of MS spectrum of *N*-benzyl *O*-ethyl thiocarbamate; and Table S 1, quantification results of four compounds in Indian cress absolute. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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